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Clinical and pathological effects of short-term cyanide repeated dosing to goats

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ABSTRACT: The purpose of this work is to determine and describe the effects of subacute cyanide toxicity to goats. Eight female goats were divided into two groups. The first group of five animals was treated with 8.0 mg KCN kg^{-1} body weight day^{-1} for seven consecutive days. The second group of three animals was treated with water as controls. Complete physical examination, including observation for behavior changes, was conducted before and after dosing. One treated animal was euthanized immediately after dosing. Later, two of the remaining treated animals and a control goat were euthanized after a 30-day recovery period. Euthanized animals were necropsied and tissues were collected and prepared for histologic studies. Clinical signs in treated goats were transient and included depression and lethargy, mild hyperpnea and hyperthermia, arrhythmias, abundant salivation, vocalizations, expiratory dyspnea, jerky movements and head pressing. Two goats developed convulsions after day 3 of treatment. One animal developed more permanent behavioral changes as she became less dominant and aggressive. Histologic changes included mild hepatocellular vacuolation and degeneration, mild vacuolation and swelling of the proximal convoluted tubules of the kidneys and spongiosis of the white matter (status spongiosis) of the cerebral white tracts, internal capsule, cerebellar peduncles, spinal cord and peripheral nerves. In summary, sub-lethal cyanide intoxication in goats resulted in behavioral changes, and during the treatment period animals showed delayed signs of toxicity. Significant histologic lesions in goats were observed and need to be characterized further. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: behavioral sequelae; central nervous system; cyanide; cyanogenic plants; goats; myelin edema; status spongiosis

Introduction

Cyanogenic plants, such cassava, sorghum and *Cynodon* grasses, have worldwide distribution and they are often ingested by animals (Tokarnia *et al*., 2000). Many poisonings are caused by consumptions of relatively high doses consumed rapidly. These poisonings have been described extensively and it has been suggested that toxicity occurs through direct inhibition of the enzyme cytochrome oxidase, resulting in cellular hypoxia and fatal cytotoxic anoxia (Ballantyne, 1987). Aware of the acute effects of cyanide poisoning, farmers have adopted management strategies to reduce such toxicity. However, the effects of such sub-acute and chronic cyanide toxicity are unknown. Association of many different human diseases with chronic cyanide exposure suggests that cyanide may cause many, yet unrecognized, problems that are of great importance for livestock kept on pastures containing cyanogenic plants.

Prolonged cyanide exposure has been associated with neurological and thyroidal disturbances in both humans and animals. Chronic cyanide exposure resulted in reduced body weight and weight gains in animals (Tewe *et al*., 1984; Ibebunjo *et al*., 1992; Soto-Blanco *et al*., 2001a). Cyanide was found to disturb thyroid metabolism in rats (Philbrick *et al*., 1979), rabbits (Ratnakumar and Rajan, 1992), pigs (Tewe *et al*., 1984), goats (Bahri, 1987; Soto-Blanco *et al*., 2001a) and humans (Adewusi and Akindahunsi, 1994). Studies in rats (Soto-Blanco *et al*., 2002a) and goats (Soto-Blanco *et al*., 2002b) chronically treated with cyanide have also described central nervous system (CNS) lesions.

The purpose of this work is to describe the effects of short-term toxicity to goats and compare them with those described in other species. Goats were chosen as the experimental species because several works evaluating cyanide toxicity in goats have been conducted in our laboratory (Soto-Blanco *et al*., 2001a,b, 2002b; Soto-Blanco and Górniak, 2003, 2004), including their toxicokinetics (Sousa *et al*., 2003).

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Materials and Methods

Animals and Experimental Design

Eight, mixed-breed, mature female goats weighing 32–50 kg were fed 100 g of concentrate and sugar cane (*Saccharum officinarum* L.) *ad libitum*. The goats were divided into two groups. The first group (five animals) was treated with 8.0 mg of KCN (Merck, Darmstadt, Germany) kg body wt^{-1} day^{-1} diluted in tap water, divided into two daily doses, by gavage for seven consecutive days. The controls received only water for the same period.

Physical examinations and behavioral assessments were conducted before and after dosing. The term 'convulsions' was used only when severe muscular contractions occurred with loss of consciousness.

Cyanide Antidote

When treated goats became severely intoxicated (animals developed severe trembling and mild convulsion), sodium nitrite (10 g 100 ml^{-1} of distilled water) at 20 mg kg body wt^{-1} and sodium thiosulfate (20 g 100 ml^{-1}) at 500 mg kg^{-1} were given i.v. as an antidote. The sodium thiosulfate treatment was repeated after 5 min.

Blood Biochemical Analysis

Blood samples were taken at the start of the study and at the end of the dosing periods and then at days 9, 10, 11 and 12. The blood samples were obtained from the jugular vein of each goat stored at −10 °C until analysis. Commercial kits were used for determination of glucose, cholesterol (Laborlab, Brazil), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), total protein, creatinine (Merck, Darmstadt) and urea nitrogen (Labtest, Brazil).

Plasma thiocyanate concentrations were determined by spectrophotometry following the method of Pettigrew and Fell (1972) but with minor modifications as described earlier (Soto-Blanco and Górniak, 2003).

Pathological Study

One treated goat (no. 1) was euthanized and necropsied immediately after the final dose. Two additional treated goats (nos 2 and 3) were euthanized and necropsied 30 days later. At necropsy the entire CNS, liver, spleen, kidneys, lungs, myocardium, pancreas, adrenal and thyroid glands were collected and fixed in Carnoy's solution. Paraffin-embedded sections were stained with H&E; CNS sections were also stained with periodic acid-Schiff (PAS)/Luxol fast blue, Bodian silver and Holzers glial fiber stain.

Statistical Analysis

Data are reported as means ± SEM and were compared using a mixed model (PROC MIXED) for a repeated-measures design (SAS Statistical Software V8, 2000; SAS Institute, Cary, NC). The level of significance was set at $P < 0.05$.

Results

Clinical signs were transient and lasted up to 1 h after KCN administration. No changes were seen in control animals. After the initial dose, the treated goats were moved slowly or were reluctant to move, with hyperpnea and increased body temperature (up to 0.5 °C). Transient tachycardia also was found in most instances but other animals were normal or had mild bradycardia.

Other clinical signs that had variable presentation included abundant salivation, vocalizations, expiratory stridor, intention tremors, jerky head movement (usually quick upwards movement of the head and neck) and head pressing.

One goat (no. 2) developed convulsions immediately after the first daily KCN dose on day 3. Antidote was given and the convulsions subsided. Similar convulsions developed after treatment on subsequent days, so the second half of this animal's dose was not administered. However, the convulsions continued to occur. Another treated goat (no. 1) developed similar convulsions after the second dose of day 3 and was also treated with antidote. The same animal developed severe muscular contractions but not convulsions after the first dose on day 6. However, it quickly recovered and the antidote treatment was not necessary. The clinical effects were variable because treated animals responded differently. One very aggressive goat that tended to hit and bully the other goats gradually became less aggressive and this behavioral change was maintained through several weeks post-treatment.

The serum biochemical results are presented in Table 1. No significant inter-group differences were detected in serum glucose, creatinine, urea or cholesterol concentrations or in the activities of AST, ALT, GGT, or alkaline phosphatase. Plasma thiocyanate concentrations (Table 2) were higher in treated goats than in controls at all evaluated days but day 0, and the highest levels were found at day 9. Thiocyanate concentrations were lower in goat no. 2 than in other treated goats because this goat only received a single cyanide dose because of its high sensitivity to cyanide.

Table 1. Biochemical panel of female goats that received zero (control group) or 8.0 mg KCN kg^{-1} day^{-1} (treated group)

	Serum components[a]							
Group	GLU	CHOL	UN	CREA	AST	ALT	γ-GT	AP
Day 1								
Control	54.0 ± 1.53	90.4 ± 5.96	34.5 ± 5.25	0.87 ± 0.02	37.7 ± 1.43	19.0 ± 1.49	21.2 ± 1.35	83.7 ± 5.53
Treated	52.4 ± 1.87	92.6 ± 9.86	22.6 ± 2.49	0.91 ± 0.03	36.9 ± 2.01	20.4 ± 1.95	21.8 ± 1.47	83.3 ± 14.0
Day 7								
Control	48.3 ± 1.86	84.8 ± 8.59	35.1 ± 2.91	0.91 ± 0.02	36.7 ± 0.54	20.0 ± 2.35	20.6 ± 1.35	75.2 ± 4.84
Treated	50.2 ± 2.92	91.8 ± 10.4	32.6 ± 0.90	0.91 ± 0.06	36.2 ± 1.59	23.0 ± 1.29	22.6 ± 0.92	61.3 ± 10.8
Day 9								
Control	52.8 ± 1.50	89.1 ± 19.3	23.3 ± 3.63	0.91 ± 0.02	36.4 ± 2.43	17.0 ± 0.81	20.8 ± 1.16	78.9 ± 2.06
Treated	50.3 ± 2.09	87.3 ± 14.0	22.2 ± 1.00	1.07 ± 0.12	36.8 ± 1.02	19.0 ± 0.78	22.3 ± 1.47	61.2 ± 13.3
Day 30								
Control	52.1 ± 1.85	90.0 ± 16.7	41.2 ± 2.95	0.94 ± 0.01	42.1 ± 0	17.0 ± 0.81	23.4 ± 0.29	67.8 ± 4.13
Treated	45.7 ± 3.49	93.6 ± 9.10	34.7 ± 3.11	0.97 ± 0.08	42.5 ± 0.77	21.4 ± 1.79	23.3 ± 0.96	59.5 ± 6.19

[a] Values are means ± SEM. GLU, glucose (mg dl^{-1}); CHOL, cholesterol (mg dl^{-1}); UN, urea nitrogen (mg dl^{-1}); CREA, creatinine (mg dl^{-1}); AST, aspartate aminotransferase (U l^{-1}); ALT, alanine aminotransferase (U l^{-1}); γ-GT, γ-glutamyltransferase (U l^{-1}); AP, alkaline phosphatase (U l^{-1}).

Table 2. Plasma thiocyanate levels of female goats that received zero (control group) or 8.0 mg KCN kg^{-1} day^{-1} (treated group)

Period	Control[a]	Treated[a]	Goat no. 2
Day 0	7.56 ± 0.34	7.04 ± 0.40	7.56
Day 7	7.56 ± 0.34	125.6 ± 3.89*	79.4
Day 9			
Morning	8.40 ± 0.24	145.9 ± 31.6*	17.2
Afternoon	7.77 ± 0.17	103.5 ± 20.8*	14.7
Day 10			
Morning	8.61 ± 0.26	28.0 ± 9.55*	9.24
Afternoon	7.14 ± 0.21	21.3 ± 6.49*	9.24
Day 11			
Morning	8.19 ± 0.17	15.1 ± 6.00*	7.14
Afternoon	8.82 ± 0.51	13.4 ± 3.38*	8.34
Day 12	8.61 ± 0.17	11.5 ± 1.49*	8.08

[a] Values are means ± SEM; * $P < 0.05$, ANOVA followed by Duncan's test.

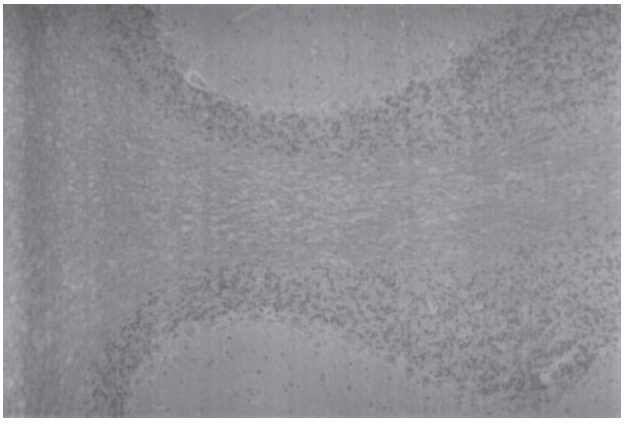

Figure 1. Cerebellar white matter showing spongiosis from a goat that received KCN for 7 days (H&E, 100×)

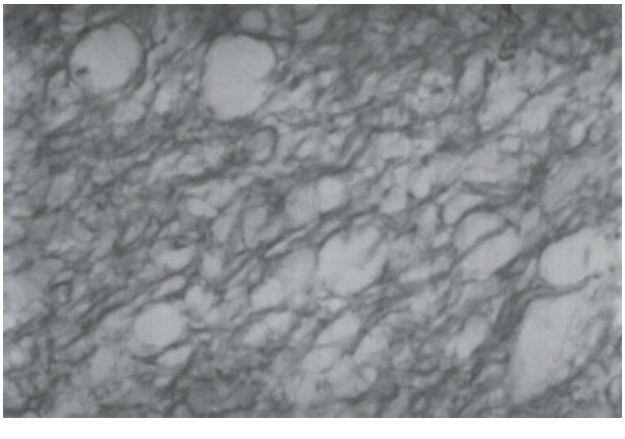

Figure 2. Spongiosis on cerebellar white matter of a goat treated with KCN for 7 days (H&E, 1000×)

No significant gross lesions were found in any of the necropsied goats. Additionally, no microscopic changes were found in the control goat. In the treated animals, histologic changes included minimal hepatocellular vacuolation and swelling. This was most severe in periportal hepatocytes. The kidneys also had minimal vacuolation and swelling of the epithelial cells of the proximal convoluted tubules. The most striking lesion was that of mild spongiosis of the white matter of the cerebral white tracts, internal capsule and cerebellar peduncles (Figs 1 and 2). Occasionally, similar mild lesions were also present in the white tracts of the spinal cord and peripheral nerves.

Discussion

It has been suggested that the constant exposure to cyanide could increase the tolerance of the organism to its toxicity (D'Mello, 1987; Tokarnia *et al*., 2000). On the other hand, the goats in this study did not present this tolerance but they presented delayed signs of cyanide poisoning. Similar delayed signs were observed in goats treated with KCN for >120 days (Soto-Blanco *et al*., 2001a), and in sheep that were treated with the

cyanogenic glucoside amygdalin (J. J. Villalba and F. D. Provenza, personal communication). This may be due to exhaustion of the mechanism of cyanide detoxification. A key step in cyanide detoxification is catalyzed by rhodanese because cyanide is bound irreversibly to sulfur from compounds such as thiosulphate and polythionates, producing thiocyanate. Although rhodanese is recycled and large amounts of cyanide can be metabolized, both cyanide and sulfite can inhibit rhodanese activity in the absence of thiosulfate (Bhatt and Linnell, 1987). Thus, the exposure to high, but not lethal, cyanide doses could deplete endogenous sulfur pools and partially impair cyanide detoxification. Another possibility is a neuronal accumulation of cyanide, which was observed earlier in *in vitro* studies (Borowitz *et al*., 1994).

Long-term cyanogenic plant consumption by both men (Adewusi and Akindahunsi, 1994) and animals (Bahri, 1987; Kamalu and Agharanya, 1991) has been associated with the development of hypothyroidism. Thiocyanate, the main cyanide metabolite, is thought to be responsible because it competes with iodide in the thyroid gland (Delange and Ermans, 1996). Previous work with rats (Sousa *et al*., 2002) and goats (Soto-Blanco *et al*., 2001a) verified that the prolonged administration of KCN caused an increase in the number of resorption vacuoles in the follicles of the thyroid. However, no alterations were found in the thyroid glands of the treated animals in this study. This is likely to be due to the relatively short dosing period.

Tropical diabetes, or malnutrition-related diabetes mellitus, is a subclass of diabetes that has been associated with chronic cyanide exposure through consumption of cassava (McMillan and Geevarghese, 1979; Petersen, 2002). However, some researchers have not been able to reproduce cassava's diabetogenic effects using cyanide alone (Kamalu, 1991; Okolie and Osagie, 2000; Soto-Blanco *et al*., 2001b). It may be that cassava contains a second diabetogenic toxin, or that this effect is a result of cyanide synergism with some contributing factor. Furthermore, epidemiological surveys in humans did not detect a relationship between cassava feeding and the development of pancreatic diabetes (Teuscher *et al*., 1987; Vannasaeng *et al*., 1988; Narendranathan and Cheriyan, 1994). This is probably a complex question and more studies are needed to determine fully whether cassava contains a yet unidentified diabetogenic toxin or is not involved in the etiology of tropical diabetes.

Nephrosis has been described also in rats (Ononogbu and Emole, 1978), dogs (Kamalu, 1993) and humans (Clark, 1936) fed cassava. Cassava treated dogs also developed periportal hepatocellular vacuolation (Kamalu, 1993). Recently Okolie and Osagie (1999) and Sousa *et al*. (2002) described similar degenerative changes in the liver and kidney from rabbits and rats treated with KCN. The goats in this study had minimal changes in both the liver and kidney that were not supported by the changes in serum biochemical markers of liver and kidney disease. It is likely that they are not clinically significant at these doses and durations. Additional work is needed to determine if cyanide is nephro- and hepatotoxic to goats at higher doses or longer durations.

The main target system for cyanide's toxicity is inhibition of cellular respiration. The CNS is especially sensitive to many toxins that affect respiration. Neurons have relatively high metabolic rates with little capacity for anaerobic metabolism (D'Mello, 1987). Cyanide also may be directly neurotoxic. It has been shown to interfere with several neurotransmitters including γ-aminobutyric acid (GABA), glutamic acid (Cassel *et al*., 1991), acetylcholine (Owasoya and Iramain, 1980), dopamine (Cassel *et al*., 1995), excitatory amino acids and nitric oxide (Gunasekar *et al*., 1996). Furthermore, Borowitz *et al*. (1997) suggested that cyanide also would be an endogenous neurotransmitter.

The change of behavior seen in one treated goat in this study was interesting, and to our knowledge no similar finding has been described in livestock. Some sequelae of cyanide toxicity in humans have been associated with damage to the basal ganglia, including Parkinsonian symptoms (Finelli, 1981; Uitti *et al*., 1985; Carella *et al*., 1988; Rosemberg *et al*., 1989; Feldman and Feldman, 1990; Messing, 1991), phobic anxiety (Nicholson and Vincenti, 1994) and psychosis (El Ghawabi *et al*., 1975; Kales *et al*., 1997). Furthermore, cyanide intoxication has been linked to personality change in a man (Uitti *et al*., 1985). In rodents, motor and cognitive function (D'Mello, 1987) and memory (Mathangi and Namasivayam, 2000) have been hypothesized as being affected by cyanide.

The vacuolation of neuropil (status spongiosis) seen in the myelin tracts in the cerebral and cerebellar white matter is suggestive of myelenic edema. Additional work, including electron microscopy, is need to characterize better this lesion. In an earlier experiment, goats chronically treated with KCN developed axonal dystrophy (spheroids) and spongiosis and gliosis in the medulla oblongata (Figs 3 and 4), gliosis in the pons and Purkinje cell degeneration in the cerebellum (Soto-Blanco *et al*., 2002b). The differences in pathological findings are likely to be due to differences in dose, duration and physiological status, because the earlier study used growing male goats that received lower doses of KCN (up to 3.0 mg kg^{-1} day^{-1}) for 5 months (Soto-Blanco *et al*., 2002b).

Status spongiosis is the term applied to the appearance of spongy nervous tissue on light microscopy (Adornato and Lampet, 1971). Various spongiform myelinopathies in ruminants (similar to found in our goats) were described as resulting from poisoning by hexachlorophane (Hall and Reid, 1974), including: *Stypandra glauca* (Whittington *et al*., 1988), *S. imbricata* (Main *et al*., 1981), *Diplodia maydis* (Prozesky *et al*., 1994) and *Helichrysum argyrosphaerum* (Van der Lugt *et al*., 1996). Additional

Figure 3. Medulla oblongata of a goat that received KCN for 7 days, showing severe spongiosis on white tracts (H&E, 100×)

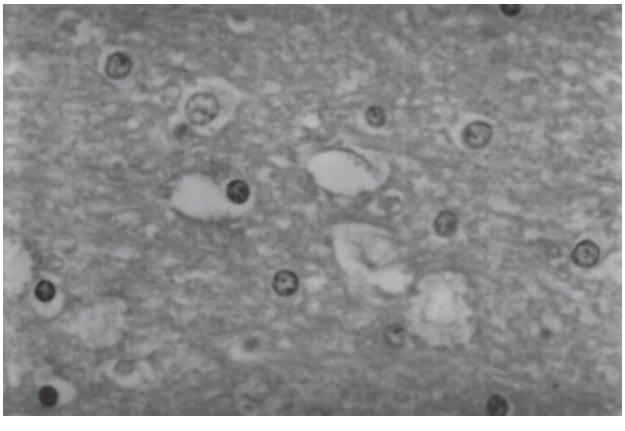

Figure 4. Spongiosis on white tracts in the medulla oblongata of a goat treated with KCN for 7 days (H&E, 1000×)

comparative and mechanistic studies are needed for better characterizeation of these lesions, to determine how they develop and if the process is reversible.

In summary, we found that sub-lethal cyanide doses given repeatedly for 7 days cause significant clinical and neurologic lesions. Such cyanide exposure is certainly dangerous and is likely to compromise significantly the animal's health.

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